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On October 30, 2001

TOWNSEND and TOWNSEND and CREW LLP

By:

PATENT
Attorney Docket No.: 14907-001900US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Joe Buechler *et al.*

Application No.: 09/158,180

Filed: September 21, 1998

For: DIAGNOSTIC ASSAYS FOR DETECTION OF *CRYPTOSPORIDIUM PARVUM*

Examiner: Hines, J.

Art Unit: 1645

Declaration Of Dr. Gunars E. Valkirs under 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Gunars E. Valkirs, Ph.D., declare as follows:

1. I am one of the co-inventors of the invention disclosed and claimed in the above-referenced patent application. I am currently Vice President of Biosite Discovery at Biosite Incorporated (Biosite). I have been involved in the development and testing of immunodiagnostic assays during the past 19 years. I have contributed to the development of various unique immunoassays for the detection of drug abuses, quantification of cardiac markers, and detection of microorganism infections.

2. To briefly describe my biography, I received my Ph.D. in physics from the University of California at San Diego. I was employed by Hybritech, Incorporated to

develop several clinical diagnostic products from 1982-88, during which I invented the ICON immunoassay system, the first immunoassay to be developed using the properties of microporous membranes to achieve both rapid and sensitive immunoassays. I co-founded Biosite in 1988 and have served as its Chief Technical Officer from its inception. At Biosite, I have participated in and overseen the development of two unique technologies that are fundamental to quantification of multiple proteins in complex samples. Specifically, the Triage® immunoassay system uses microcapillary fluidics to control the flow of fluids and fluorescent detection to enable the quantitative assay of multiple proteins in whole blood. Biosite has also developed phage display technology for the high throughput selection of high affinity antibodies from immunized mice. I am the inventor or one of the co-inventors for a number of U.S. patents resulting from the work on these technologies, namely U.S. Patent Nos. 6,207,395, 6,057,098, 5,985,579, 5,965,375, 5,939,272, 5,922,615, 5,914,241, 5,851,776, 5,679,526, 5,525,524, 5,480,792, 5,143,852, 5,089,391, and 5,028,535.

3. I have reviewed the above-referenced patent application, the Office Action dated July 5, 2001, and two references cited against the patent application, Anusz *et al.* (*J. Clinical Microbiology* 28:2770-2774, 1990) and Blunt *et al.* (*GENE*, 181:221-3, 1996). I understand that the Examiner is of the view that the kits claimed in the subject application would have been obvious in light of the results reported in these two references. I respectfully disagree with this view. My opinion is based on the following facts.

4. Traditional methods for diagnosis of *Cryptosporidium* infection involved microscopic detection of ova and parasites (O&P) in stools. Later developed tests entailed detection of surface antigens present on whole fecal oocysts. For example, Anusz *et al.* reported a method of detecting *Cryptosporidium parvum* (*C. parvum*)oocysts in stool using antibodies directed against *C. parvum* oocysts. On the other hand, protein disulfide isomerase is a soluble antigen not known to be present in stool. Soluble *C. parvum* antigens not present on the oocyst surface have not been known to be viable targets for diagnosing *C. parvum*

infection. This is because stool is rich in proteases which are expected to degrade soluble *C. parvum* antigens that are not present on the fecal oocysts.

5. Blunt et al. reported a putative PDI sequence that was identified by screening cells harboring *C. parvum* genomic DNA. Blunt et al. used an antiserum raised against a homogenate of purified *C. parvum* oocysts and sporozoites. This antiserum binds to cells harboring *C. parvum* genomic DNA under laboratory conditions. One of the positive clones thus identified contains the putative PDI sequence. There is no discussion in Blunt et al. about diagnostic methods for detecting *C. parvum* infection. Blunt et al. did not even remotely suggest that PDI is present in stool or that antibodies against a soluble PDI (or any other *C. parvum* antigen) can be used in a diagnostic assay for detecting *Cryptosporidium* in stool. The essence of the Blunt et al. report is that *C. parvum* may express a specific antigen (i.e., PDI) rather than any implication on how to diagnose *C. parvum*.

6. In my opinion, the Anusz et al. and Blunt et al. reports would not render the subject invention obvious. Anusz et al. merely described an example of the oocyst-based diagnostic tests for *C. parvum* infection. Blunt et al. only suggested that *C. parvum* express PDI, which is just one of the many soluble antigens expressed by *C. parvum*. It is one thing to know that *C. parvum* express PDI, but quite another to suggest that a soluble antigen such PDI is present in stool and suitable as target for detecting *C. parvum* in stool. Just like many other soluble antigens that are known to be expressed by *C. parvum*, the fact that *C. parvum* express PDI by no means indicates that PDI is present in stool and can be detected with an antibody against PDI. In another word, the identification of *C. parvum* PDI would not make it obvious that the prevalent oocyst surface antigen-based methods (e.g., the Anusz et al. method) should be modified by detecting a soluble antigen such as PDI in stool.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so

made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful, false statements may be jeopardize the validity of the application or any patent issuing thereon.

Date: 9/26/01

Gunars E. Valkirs, Ph.D.

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